Biol Fertil Soils (2007) 44:229–234
DOI 10.1007/s00374-007-0209-7

SHORT COMMUNICATION

Seed inoculation with *Bacillus* spp. improves seedling vigour in oil-seed plant *Jatropha curcas* L.

S. Desai · Ch. Narayanaiah · Ch. Kranti Kumari · M. S. Reddy · S. S. Gnanamanickam · G. Rajeswara Rao · B. Venkateswarlu

Received: 8 December 2006 / Revised: 27 April 2007 / Accepted: 30 May 2007 / Published online: 30 May 2007

Abstract Jatropha (*Jatropha curcas* L.) is a non-edible oil-seed plant with adaptability to marginal semi-arid lands and wastelands. The Indian Government is promoting jatropha to reduce dependence on the crude oil and to achieve energy independence by the year 2012, under the National Biodiesel Mission. Selected strains of *Bacillus* spp., either supplemented with or without chitin, were tested for their ability to promote growth of jatropha seedlings in pot culture studies. The strains supported growth of jatropha seedlings up to 42 days after sowing. Among all strains, *Bacillus pumilus* (IM-3) supplemented with chitin showed over all plant growth promotion effect resulting in enhanced shoot length (113%), dry shoot mass (360%), dry root mass (467%), dry total plant mass (346%), leaf area (256%), and chlorophyll content (74%) over control. Treating seeds with strain IM-3 without chitin resulted in enhanced dry shoot mass (473%), dry total plant mass (407%), and chlorophyll content (82%). However, *Bacillus polymyxa* (KRU-22) with chitin supported maximum root length (143%). Either strain IM-3 alone or in combination with other promising strains could be promoted further for enhanced initial seedling growth of jatropha.

S. Desai (✉) · C. Narayanaiah · C. K. Kumari · G. R. Rao · B. Venkateswarlu
Central Research Institute for Dryland Agriculture (ICAR), Santoshnagar, 500 059 Hyderabad, Andhra Pradesh, India
e-mail: desai@crida.ernet.in

M. S. Reddy
Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

S. S. Gnanamanickam
Centre for Advanced Studies in Botany, University of Madras, Chennai, Tamil Nadu, India

Keywords *Jatropha curcas* · *Bacillus* spp. · Rhizobacteria · Plant growth promotion · Seed inoculation

Introduction

Jatropha (*Jatropha curcas* L.) is a hardy non-edible oil-seed plant that can sustain harsh environments, adapt well to semi-arid marginal, and wastelands and hence has been treated as a potential alternative energy source (Foidl et al. 1996; Gubitz et al. 1999). In India, to reduce dependence on the crude oil and to achieve energy independence by the year 2012, jatropha and pongamia have been promoted under the National Biodiesel Mission. Many government and private agencies have interests in the establishment and maintenance of plantations, and the Government also supports these programmes through financial assistance. It is propagated through seeds or cuttings. Enhanced initial seedling vigour can help in better establishment of the plants especially under adverse conditions. Plant-growth-promoting rhizobacteria (PGPR) can enhance plant growth either directly by producing phytohormones or in an indirect way by producing siderophores for sequestering iron or solubilizing phosphorus or over expression of indole acetic acid (Xie Hong et al. 1996; Cattelan et al. 1999; Mayak et al. 1999). The recent review on PGPR gives an account of the current status of research in this area (Bashan and de-Bashan 2005). *Azospirillum* is one of the well-known examples of PGPR (Bashan et al. 2004). Identification of promising PGPR for jatropha helps to enhance initial seedling vigour. However, so far, no reports are available on the plant growth promotion in jatropha by using PGPR. Hence, trials were conducted to identify potential PGPR strains among *Bacillus* spp. for jatropha. Since Manjula and Podile (2001) reported that chitin supplementation resulted in enhanced

PGPR effect of *Bacillus subtilis*, strains were also evaluated for their efficacy either with or without chitin supplementation. In this paper, we report for the first time PGPR effect of strains of *Bacillus* spp. on jatropha.

Materials and methods

Among several strains, nine strains of *Bacillus* spp. viz. *B. lentus* (ALP-18), *B. polymyxa* (KRU-22), *B. polymyxa* (VLB-16), *B. coagulans* (PD-7), *B. pumilus* (IM-3), *B. cereus* (NGCI-15), *B. circulans* (VYI-18), *Bacillus* sp. (MON#-2-17), and *Bacillus* sp. (CAL-9) with a proven PGPR effect on other crops in previous studies were used (Vasudevan et al. 2002). These bacterial strains were collected from the culture collection of the Centre for Advanced Studies in Botany, University of Madras, Chennai. From the stock cultures, working cultures were prepared by inoculating a loopful of culture to 10 ml of sterile nutrient broth taken in 50 ml culture tubes and incubating at 28±1°C for 24 h. One milliliter of actively growing bacterial suspension from these tubes was used to inoculate sterile 30 ml nutrient broth in a 150-ml conical flasks at 28±1°C on a rotary shaker at 120 rpm. After 5 days of incubation, the cells were pelleted and suspended in sterile distilled water to a final concentration of 1×10^9 cells ml^{-1}. Seeds of jatropha were inoculated following the method of van Peer and Schippers (1989) with slight modifications as described in this paper. One milliliter of the bacterial suspension was thoroughly mixed with 100 mg of carboxyl methylcellulose. To this, 100 mg of fine chitin powder prepared from the chitin flakes (Sigma cat. no. C9213) was added (C^+). In another set, no chitin was added (C^-). In both cases, slurry was prepared and ten surface-sterilized seeds of uniform size were shaken well to ensure uniform coating of bacteria on seed surface and were allowed to shade-dry. Pot culture studies were conducted at the Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad. Five inoculated seeds of jatropha (cv. Local) were sown in each pot of 114×76 cm filled with 12 kg sandy loam soil and watered regularly. For each treatment, six such pots were maintained. Uninoculated seeds sown in pots served as control. For each observation, two plants were randomly selected from each treatment, and the mean of these two plants was used as one replication. The experiment was repeated twice. Observations were recorded on rate of seedling emergence, root length, shoot length, root volume, chlorophyll content, leaf area, and dry mass of root, shoot, and total plant by drawing random samples at 14th, 28th, and 42nd days after sowing (DAS). Chlorophyll content was recorded using a hand-held chlorophyll meter (Minolta SPAD-502; Krugh et al. 1994). All the percentage data were suitably transformed and analyzed in a completely randomized design (CRD). Pooled analysis of the data of both experiments was done, and the significance was tested by *F* test. For data analysis, SPSS v. 12.0 package was used.

Results and discussion

All the strains differed significantly for various growth parameters (Fig. 1). As the addition of chitin always did not result in improved performance of the strains, the interactions involving chitin were not always significant. After 10 days of sowing, in treatments IM-3(C^+), KRU-22(C^-), CAL-9(C^+) and (C^-), VYI-18(C^-), ALP-18(C^+), VLB-16 (C^-) and MON#2-17(C^+), 100% emergence was recorded, whereas only 80% emergence was recorded in control.

For root length, strains and dates differed significantly but not chitin treatments ($p=0.05$). The maximum effect of the treatments was noticed at 42 DAS in C^+ treatments of PD-7, CAL-9, and VYI-16 showing 143, 141, and 140% increase in root length over control, respectively, and all these treatments were on a par. Interestingly, KRU-22(C^-) also showed 143% increase in root length, which was on a par with PD-7(C+) treatment (Table 1).

By 42nd DAS, 60.6% increase in shoot length was recorded in IM-3(C^+) followed by 57.6 and 55.3% in VLB-16(C^+) and PD-7(C^+), respectively, and all these treatments were statistically on a par. Although only C^+ treatments of KRU 22, NGCI-15, and Cal-9 showed enhanced shoot length at 14DAS, their effect did not sustain until 42 DAS (Table 1). Even a reduction of 23.5% at 14 DAS was recorded in Mon# 2-17(C^-) treatment.

The leaf area of treated plants increased significantly across dates of sampling, which ranged from 72.5 to 672.1 cm^2 (SD=±163.29) in C^+ treatments and 72.5 to 550.7 cm^2 (SD=±133.04) in C^- treatments ($p=0.05$). At 42 DAS, IM-3 (C^+) showed maximum increase in leaf area (256%) followed by PD-7(C^+), which showed 233% increase. All strains responded to chitin supplementation

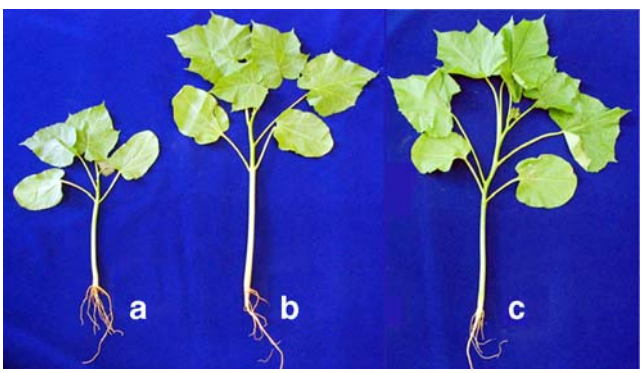

Fig. 1 Effect of *Bacillus pumilus* (IM-3) on overall plant growth of *Jatropha curcas* (*a* control, *b* with chitin, *c* without chitin)

Table 1 Effect of seed inoculation with different isolates of *Bacillus* spp. on root- and shoot-length of jatropha after four dates of sowing

Treatment	Root length							Shoot length						
	14DAS		28DAS		42DAS		S. Ed±, CD(0.05)	14DAS		28DAS		42DAS		S. Ed±, CD(0.05)
	C^+	C^-	C^+	C^-	C^+	C^-		C^+	C^-	C^+	C^-	C^+	C^-	
IM-3	11.0[a]	9.8	21.0	20.0	28.8	28.9		20.2	20.1	25.5	22.5	27.3	23.9	
KRU-22	9.8	13.9	20.0	24.7	32.9	29.7		23.5	20.3	22.9	23.2	25.7	22.1	
NGCI-15	8.0	10.3	16.8	19.1	26.9	30.9		27.8	21.5	22.3	22.7	22.0	23.1	
CAL-9	6.5	7.2	16.6	16.2	28.5	32.5		24.9	21.5	22.1	22.0	23.3	23.3	
PD-7	12.4	13.5	15.7	21.4	26.2	32.9		20.6	20.7	23.9	23.2	26.4	24.7	
VYI-18	12.5	11.9	21.3	20.0	23.5	32.5		19.9	21.3	23.0	23.5	20.3	24.6	
ALP-18	10.8	10.7	14.9	17.2	23.2	27.8		20.8	19.8	25.8	21.4	20.3	21.9	
VLB-16	12.1	6.5	18.0	14.0	25.8	19.9		21.5	19.5	27.7	22.6	26.8	24.0	
MON#2-17	11.0	10.8	16.6	17.1	29.5	24.5		21.4	15.3	23.6	21.2	23.3	21.9	
Control	8.7	8.7	13.9	13.9	13.5	13.5		20.0	20.0	16.3	16.3	17.0	17.0	
Strain(S)							1.39, 2.77							1.12, 2.25
Chitin(C)							0.62, NS							0.50, 1.01
Dates(D)							0.76, 1.52							0.62, 1.23
SxC							1.96, 3.92							1.59, NS
SxD							2.40, 4.81							1.95, 5.18
CxD							1.07, NS							0.87, NS
SxCxD							3.40, NS							2.75, NS
CV%							18.58							12.41

C^+ With chitin, C^- without chitin

[a] Pooled mean two trials with three replications each

(Table 2). Throughout experiment, there was an increase in leaf area across the treatments.

In all the treated plants, there was an increase in root volume ranging from 1.25 to 3.75 cm^3. The highest root volume of 3.75 cm^3 after 42 DAS was observed in the plants treated with IM-3 (C^-) followed by 3.50 $cm^3$and in IM-3 (C^+), which were statistically at par, whereas in control the root volume was only 0.70 cm^3. In other

Table 2 Effect of different isolates of *Bacillus* spp. on leaf area and chlorophyll content of jatropha after four dates of sowing

Treatment	Leaf area (cm^2)							Chlorophyll content ($\mu g cm^{-2}$)						
	14DAS		28DAS		42DAS		S. Ed±, CD(0.05)	14DAS		28DAS		42DAS		S. Ed±, CD(0.05)
	C^+	C^-	C^+	C^-	C^+	C^-		C^+	C^-	C^+	C^-	C^+	C^-	
IM-3	206	165	439	343	672	523		113	118	203	183	264	276	
KRU-22	197	190	397	383	535	662		137	106	290	212	242	203	
NGCI-15	214	210	371	340	332	504		116	110	206	196	202	222	
CAL-9	173	205	308	292	471	523		111	114	185	180	238	225	
PD-7	199	202	499	430	629	654		106	117	211	213	255	236	
VYI-18	191	211	440	420	365	346		111	114	222	228	216	184	
ALP-18	208	188	430	309	386	491		106	108	207	175	207	168	
VLB-16	223	185	573	272	537	582		113	113	226	167	257	189	
MON#2-17	168	168	415	276	389	449		123	107	187	174	212	182	
Control	73	73	147	147	189	287		134	134	135	135	152	152	
Strain(S)							36.81, 73.6							11.3, 22.7
Chitin(C)							16.5, 32.9							5.1, 10.1
Dates(D)							20.2, 40.3							6.2, 12.4
SxC							52.1, NS							16.0, NS
SxD							63.8, 127.5							19.6, 52.3
CxD							28.5, 57.0							8.8, NS
SxCxD							90.2, NS							27.8, NS
CV%							28.24							16.00

C^+ With chitin, C^- without chitin

Table 3 Effect of different strains of *Bacillus* spp. on dry shoot mass (g) and dry total plan mass (g) of jatropha after different dates after sowing

Treatment	Dry root mass (g)							Dry shoot mass (g)							Dry total plant mass (g)						
	14 DAS		28 DAS		42 DAS		S. Ed±, CD(0.05)	14 DAS		28 DAS		42 DAS		S. Ed±, CD(0.05)	14 DAS		28 DAS		42 DAS		S. Ed±, CD(0.05)
	C^+	C^+	C^-	C^+	C^-	C^+		C^-	C^-	C^+	C^-	C^+	C^-		C^-	C^-	C^+	C^-	C^+	C^-	
IM-3	0.05	0.05	0.30	0.20	0.85	0.85		0.35	0.40	2.05	1.90	3.45	4.30		0.85	0.85	3.40	3.10	6.25	7.10	
KRU-22	0.04	0.07	0.25	0.31	0.55	0.55		0.45	0.41	1.85	2.20	3.35	2.85		0.99	0.93	3.00	3.51	5.45	4.50	
NGCI-15	0.05	0.05	0.21	0.22	0.45	0.55		0.50	0.50	1.80	1.90	2.30	2.80		0.95	1.10	2.96	2.82	3.70	4.60	
CAL-9	0.05	0.07	0.19	0.16	0.50	0.65		0.45	0.45	1.55	1.60	2.85	4.15		0.90	1.02	2.44	2.46	4.80	6.15	
PD-7	0.04	0.08	0.25	0.26	0.60	0.60		0.35	0.35	2.00	1.95	3.30	3.55		0.84	0.93	3.75	3.31	5.55	5.45	
VYI-18	0.03	0.06	0.22	0.25	0.35	0.45		0.45	0.45	1.70	1.80	2.30	2.90		0.93	0.96	3.02	2.95	3.75	4.45	
ALP-18	0.05	0.05	0.23	0.2	0.45	0.40		0.45	0.45	2.15	1.50	2.10	2.70		0.95	0.95	3.48	2.45	3.65	4.20	
VLB-16	0.07	0.05	0.22	0.15	0.4	0.30		0.45	0.45	2.10	1.10	2.20	2.10		0.97	0.90	3.67	1.80	4.15	3.35	
MON#2-17	0.05	0.05	0.18	0.15	0.35	0.35		0.50	0.45	1.65	1.25	2.40	2.15		0.90	0.90	2.78	1.95	3.75	3.25	
Control	0.03	0.03	0.08	0.08	0.15	0.15		0.20	0.20	0.60	0.60	0.75	0.75		0.43	0.43	0.93	0.93	1.40	1.40	
S							0.04, 0.07							0.16, 0.31							0.21, 0.43
C							0.02, NS							0.07, NS							0.10, NS
D							0.02, 0.05							0.09, 0.23							0.12, 0.31
SxC							0.05, NS							0.22, NS							0.30, 0.81
SxD							0.06, 0.16							0.27, 0.72							0.37, 0.99
CxD							0.03, NS							0.12, 0.32							0.17, 0.44
SxCxD							0.09, NS							0.38, NS							0.53, NS
CV%							35.28							19.74							24.34

C^+ With chitin, C^- without chitin, *S* strain, *C* chitin, *D* date

isolates, response varied over sampling dates for their ability to enhance root volume. However, plants treated with PD-7, CAL-9, NGCI-15, KRU-22, ALP 18, and MON #2-17 (all with or without chitin) and VYI-18(C^-) showed a constant increase in root volume at all the sampling dates.

For chlorophyll content, while strains, dates, chitin treatments, and interaction of strains and dates differed significantly over control, other interactions were not significant (p=0.05). The chlorophyll content in C^+ treatments ranged from 111.0 to 337.5 μgcm^{-2} (SD=±61.49), and in C^- treatments, it ranged from 105.6 to 323.5 μgcm^{-2} (SD=±133.04). A maximum increase of 74 and 82% chlorophyll content was recorded in IM-3(C^+) and IM-3 (C^-) treatments, respectively (Table 2). Interestingly, at 14 DAS in all the treatments, there was a negative effect ranging from 8 to 21% except in KRU-22(C^+).

All the bacterial treatments and dates of sampling differed significantly for root dry mass over control (p=0.05). The root dry mass across C^+ treatments ranged from 0.03 to 1.02 g (SD=±0.297) and across C^- treatments ranged from 0.03 to 1.06 g (SD=±0.296). Substantial increase of >200% in dry mass of root was recorded in 13 each of C^+ and C^- treatments. Only VYI-18(C^+) treated plants at 14 DAS were at par with control. At 42 DAS, as high as 467% increase in dry root mass was recorded in both IM-3 (C^+ and C^-) treatments. All treated plants showed a constant increase in root dry-mass at all the sampling dates, except C^+ and C^- treatments of ALP-18 and Mon#2-17 (Table 3).

The shoot dry mass across C^+ treatments ranged from 0.20 to 4.55 g (SD=±1.207) and across C^- treatments ranged from 0.20 to 5.03 g (SD=±1.309). IM-3 (C^+) and IM-3 (C^-) showed maximum dry shoot mass at 42 DAS, which was 360 and 473%, respectively (Table 3). Out of 36 C^- treatments, 13, 14, and 9 showed increase in dry shoot mass of >200, 100–200, and <100, respectively. Similarly, in C^+ treatments, 12, 18, and 6 treatments showed increased shoot dry mass of >200, 100–200, and <100%, respectively.

The strains differed significantly for dry total plant mass at different sampling dates (p=0.05), and a substantial increase (407% over control) was recorded in IM(C^-) at 42 DAS followed by 346% in IM-3(C^+). In C^+ treatments, the increase ranged from 0.85 to 6.25 g (±1.944). Similarly, in C^- treatments, it ranged from 0.85 to 7.10 g (±2.015).

Deka Boruah et al. (2003) demonstrated that seed inoculation with *Pseudomonas* improved germination, shoot length, root length, dry mass, enhanced yield, and chlorophyll content of leaves of *Phaseolus vulgaris*. Inoculation of maize seeds with *Pseudomonas* strains GRP3A and PRS1 significantly increased the germination percentage, maximum shoot and root length, and dry mass (Sharma et al. 2003). Similar results have been reported by Bashan et al. (2006) when wheat seedlings inoculated with *Azospirillum brasiliense* showed significantly increased quantity of several photosynthetic pigments, such as chlorophyll a, chlorophyll b, and the auxiliary photoprotective pigments, such as violaxanthin, zeaxanthin, antheroxanthin, lutein, neoxanthin, and β-carotene. The extensive review on *Azospirillum* elucidates its plant-growth-promoting ability through production of phytohormones and improvement of absorption of water and minerals in several crop plants (Bashan et al. 2004). Ma et al. (1995) have identified leaf greenness as an indirect indicator of the photosynthetic rate of soybean. In the present study, also in some treatments, the chlorophyll meter readings were significantly high as compared to control indicating more greenness that could help in enhanced photosynthetic rate. Samdur et al. (2000) have used chlorophyll meter for screening groundnut genotypes for tolerance of iron deficiency chlorosis. The slight negative impact on chlorophyll content at 14 DAS indicates thin distribution of the chlorophyll content that could be due to formation of tissues that support the vigour of the plant at the initial phenophase rather than photosynthetic apparatus. Similarly, a reduction in shoot length at 14 DAS in Mon# 2-17(C^-) could be due to its deleterious effect on seedlings. Supplementing with chitin has been reported to enhance biocontrol ability of *B. subtilis* AF1 (Kishore et al. 2005). However, in this study, chitin supplement did not have a profound effect on growth promotion ability of the strains. The overall improvement in seedling vigour through a significant increase in various physiological parameters suggests that these *Bacillus* strains have a plant-growth-promoting ability on jatropha seedlings and hence could be used for seed inoculation for better establishment of seedlings. The plants with enhanced seedling vigour can help in better establishment of plantations. As the seeds of jatropha contain about 35% oil, which after esterification can be mixed with crude oil up to 20% without any modifications to the functionality of the engines (Rao and Gopalakrishnan 1991; Saka 1991), the energy demands of the growing populations could be met through these biofuels and sustain economies of developing nations.

Acknowledgment The authors are thankful to the director of CRIDA for his constant encouragement and logistic support.

References

Bashan Y, de-Bashan LE (2005) Bacteria/plant growth promotion. In: Hillel D (ed) Encyclopedia of soils in the environment, vol. 1. Elsevier, Oxford, UK, pp 103–115

Bashan Y, Holguin G, de-Bashan LE (2004) Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Can J Microbiol 50:521–577

Bashan Y, Bustillos JJ, Leyva LA, Hernandez JP, Bacilio M (2006) Increase in auxiliary photoprotective photosynthetic pigments in

wheat seedlings induced by *Azospirillum brasilense*. Biol Fertil Soils 42:279–285

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680

Deka Boruah HP, Rabha BK, Saikia N, Dileep Kumar BS (2003) Fluorescent *Pseudomonas* influences palisade mesophyll development and spatial root development in *Phaseolus vulgaris*. Plant Soil 256:291–301

Foidl N, Foidl G, Sanchez M, Mittelbach M, Hackel S (1996) *Jatropha curcas* L. as a source for the production of biofuel in Nicaragua. Bioresour Technol 58:77–82

Gubitz GM, Mittelbach M, Trabi M (1999) Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresour Technol 67:73–82

Kishore GK, Pande S, Podile AR (2005) Chitin-supplemented foliar application of *Serratia marcescens* GPS 5 improves control of late leaf spot disease of groundnut by activating defence-related enzymes. J Phytopathol 153:169–173

Krugh B, Bickham L, Miles D (1994) The solid-state chlorophyll meter: a novel instrument for rapidly and accurately determining the chlorophyll concentrations in seedling leaves. Maize Genet Coop News Lett 68:25–27

Ma BL, Morrison MJ, Voldeng HD (1995) Leaf greenness and photosynthetic rates in soybean. Crop Sci 35:1411–1414

Manjula K, Podile AR (2001) Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF 1. Can J Microbiol 47:618–625

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18:49–53

Rao PS, Gopalakrishnan KV (1991) Vegetable oils and their methyl esters as fuels for diesel engines. Indian J Technol 29:292–297

Saka S (1991) Biodiesel fuel. In: Saka S (ed) Biomass, energy and environment. Industrial Publishing, Japan, pp 454–456

Samdur MY, Singh AL, Mathur RK, Manivel P, Chikani BM, Gor HK, Khan MA (2000) Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. Curr Sci 79:211–214

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894

van Peer R, Schippers B (1989) Plant growth responses to bacterization with selected *Pseudomonas* spp. strains and rhizosphere microbial development in hydroponic cultures. Can J Microbiol 35:456–463

Vasudevan P, Reddy MS, Kavitha S, Velusamy P, David Paulraj RS, Purushothaman SM, Brinda Priyadarsini V, Bharatkumar S, Kloepper JW, Gnanamanickam SS (2002) Role of biological preparations in enhancement of rice seedling growth and grain yield. Curr Sci 83:1140–1143

Xie Hong, Pasternak JJ, Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr Microbiol 32:67–71